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Effect of cotton nitrogen fertilization on *Bemisia argentifolii* populations and honeydew production

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Abstract

The impact of nitrogen fertilization on cotton plants, *Gossypium hirsutum* L., silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, population dynamics and honeydew production were investigated in the field at Riverside, California, USA. Treatments were soil applications of 0, 112, 168 and 224 kg nitrogen per hectare, and a soil application of 112 kg of nitrogen plus a foliar application of 17 kg nitrogen per hectare. Increased numbers of both adult and immature whiteflies occurred during population peaks with increasing amounts of applied nitrogen. Higher numbers of whiteflies resulted in increased levels of honeydew. Increasing plant nitrogen also enhanced cotton foliar photosynthetic rates and stomatal conductance, and altered concentrations of glucose, fructose and sucrose in cotton petioles. However, at our treatment levels nitrogen had no effect on seedcotton yield. Petiole glucose levels were significantly correlated with numbers of whitefly adults on leaves during their peak populations. Significant correlations between whitefly numbers and other cotton physiological parameters occurred on only a few sampling dates.

Introduction

Dietary nitrogen and carbohydrates impact survival, growth and reproduction of insects (White, 1984; Joern & Behmer, 1997). Plant nitrogen fertilization has been shown to modify the dietary nitrogen concentration of the plants for phloem-feeding insects to affect their population growth (Jasson & Smilowitz, 1986; Jauset et al., 1998; Godfrey & Leser, 1999). However, the effect of nitrogen fertilization on plant carbohydrates and the related population growth of the insects are still poorly understood.

The only sugar in cotton phloem sap is sucrose (Tarczynski et al., 1992). Phloem-feeding insects such as the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, convert much of the sucrose they ingest into various oligosaccharides which are secreted as droplets of a concentrated syrup called honeydew. Silverleaf whitefly honeydew contains several dozens of carbohydrates, particularly sucrose, melezitose, fructose, glucose and trehalulose (Hendrix et al., 1992; Hendrix, 1999; Hendrix & Wei, 1994). Honeydew sugars are created from dietary sucrose as a means of regulating the osmotic gradient between the gut lumen and hemolymph in phloem-feeding insects (Fisher et al., 1984; Rhodes et al., 1997). The most abundant sugar in the whitefly honeydew is the disaccharide trehalulose (Hendrix et al., 1992; Tarczynski et al., 1992). A number of unusual sugars have been discovered in whitefly honeydew and in their hemolymph which may be involved in such osmoregulation (Hendrix & Wei, 1994; Wei et al., 1996, 1997; Hendrix & Salvucci, 2000). However, a causal relationship between carbohydrate metabolism in host plants and whitefly metabolism has not been elucidated.

The silverleaf whitefly is a major pest of cotton and other crops. Large populations of this insect can ingest sufficient quantities of plant phloem sap to cause yield reductions (Gerling et al., 1980; Bellows & Arakawa, 1988; Henneberry et al., 1995). In addi-

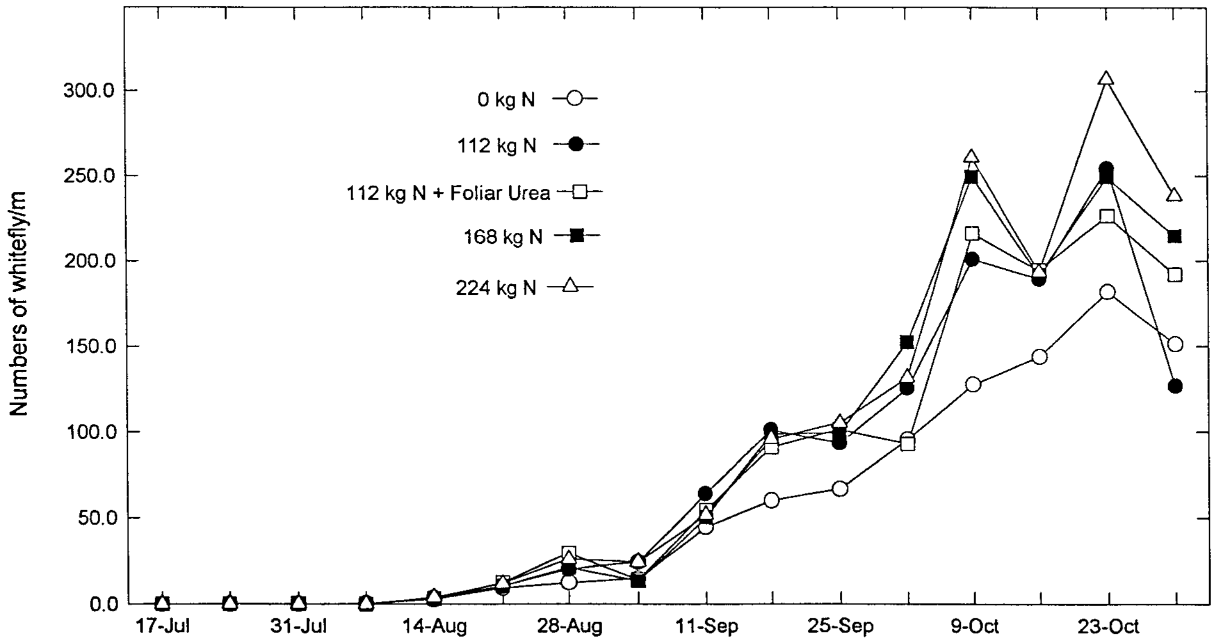

Figure 1. Effect of nitrogen fertilizer treatments on numbers of adult whiteflies on cotton.

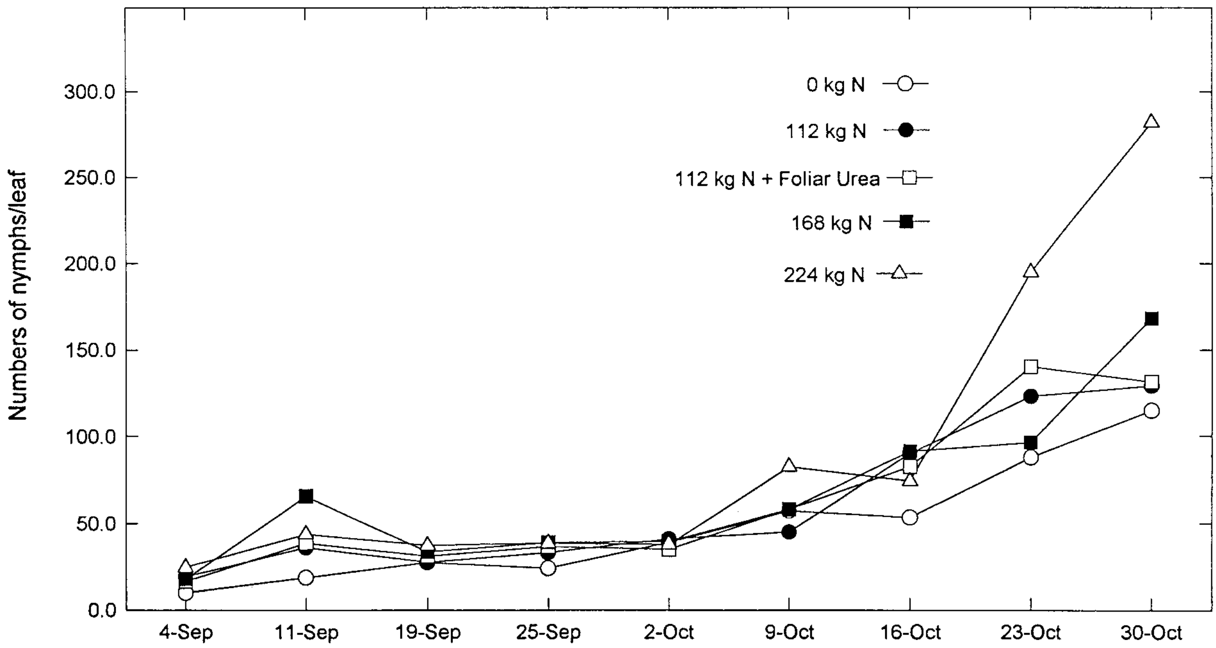

Figure 2. Effect of nitrogen fertilizer treatments on numbers of immature whiteflies on cotton.

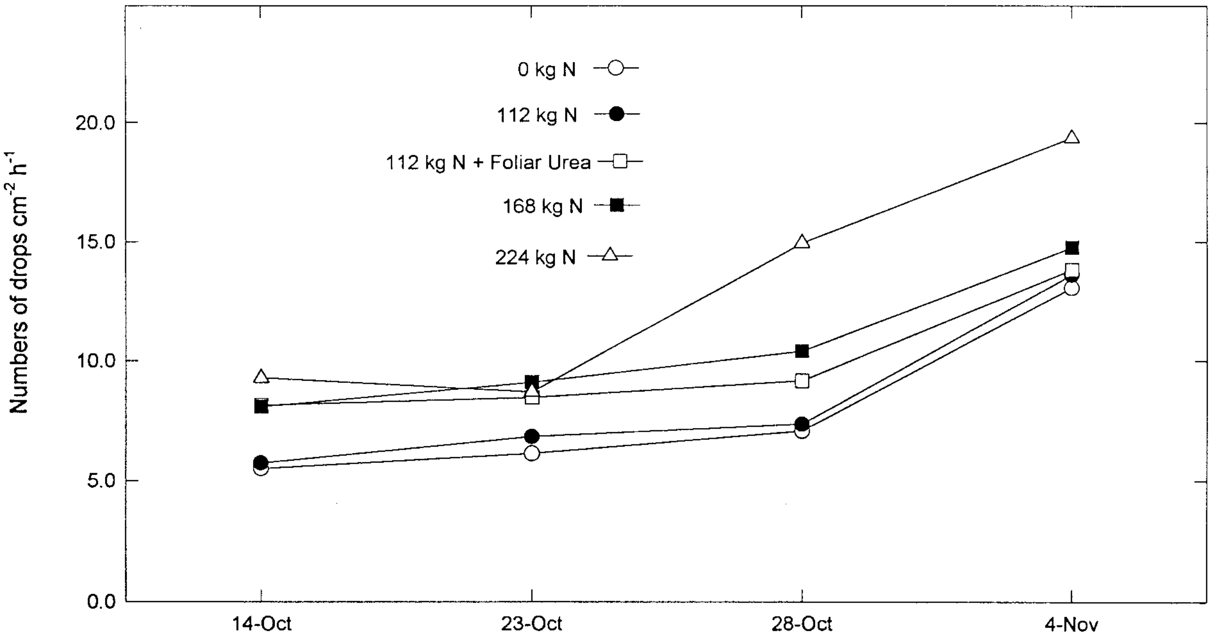

Figure 3. Effect of nitrogen fertilizer treatments on numbers of honeydew drops produced by whiteflies.

tion, whitefly honeydew secreted can fall on cotton lint to produce 'sticky' cotton, that causes problems during lint processing at textile mills (Perkins, 1986; Henneberry et al., 1996). The honeydew deposited on leaves provides a suitable substrate for sooty mold development, which inhibits foliar photosynthesis (Yee et al., 1996).

Plant nitrogen fertilization effects on whiteflies and honeydew production under greenhouse conditions have been reported by several researchers (Blua & Toscano, 1994; Rubeiz et al., 1995; Bentz et al., 1995). Blua & Toscano (1994) indicated subtle differences in silverleaf whitefly development at different levels of cotton nitrogen fertilization. On higher nitrogen treated plants, early-instar whiteflies initiated production of honeydew earlier than those on plants treated with medium or low nitrogen but subsequently generated fewer droplets (Blua & Toscano, 1994). Rubeiz et al. (1995) reported that there were no significant differences in populations of the sweetpotato whitefly, *B. tabaci* (Gennadius), between the control and the nitrogen fertilized cantaloupes. However, Bentz et al. (1995) found more *B. tabaci* on fertilized poinsettia plants than on nonfertilized controls. The effects of nitrogen fertilization on plant-whitefly interactions under field conditions have not been fully investigated. The present study was initiated to determine if different levels of nitrogen fertilization to cotton plants grown in field increased whitefly numbers and honeydew production, and to determine the related carbohydrate-based biochemical and physiological mechanisms.

Materials and methods

Experimental plots. Cotton (*Gossypium hirsutum*, cv. Acala) was planted on 20 May 1998 at the Agricultural Experimental Station, University of California, Riverside. Five nitrogen levels were evaluated in a randomized complete block design with five replicates. The plot size was 16 m long and 8 m wide with 3 m buffer areas between neighboring plots in the same block. Each of the five blocks was separated by four rows of bare soil. Row spacing was 1 m and there were eight rows in each plot. Plants were thinned to 10 cm interval spacing at the 4 node stage of plant development. Treatments consisted of soil applications of 0, 112, 168, and 224 kg of urea nitrogen per hectare, and one treatment combining a soil application of 112 kg of urea nitrogen and a foliar application of 17 kg of urea nitrogen per hectare. These treatments represented sub-optimal, optimal, and supra-optimal nitrogen fertility for cotton in this field. Soil applications of nitrogen were side-dressed when the plants were at the 7 node growth stage (6 July). The foliar

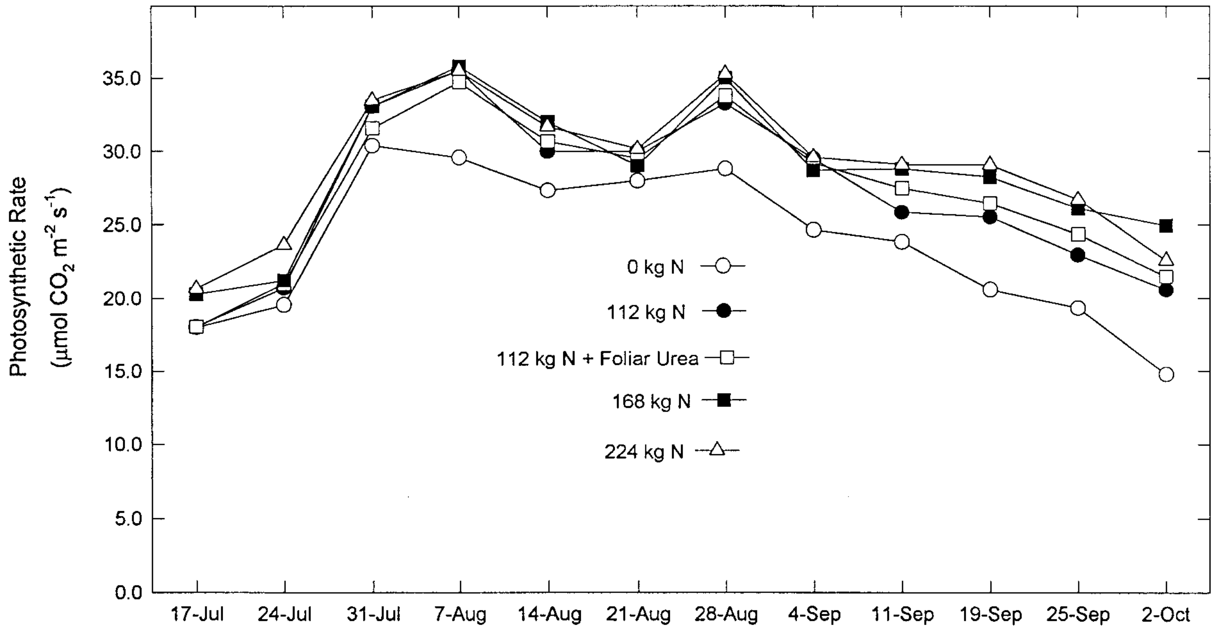

Figure 4. Effect of nitrogen fertilizer treatments on cotton foliar photosynthetic rate.

nitrogen was applied with a hand sprayer just prior to flowering (27 August). Prior to planting, five soil samples (within 15 cm of top soil) across each experimental plot were analyzed to determine residual total nitrogen.

The field was furrow-irrigated every 2 weeks prior to nitrogen fertilization and every week thereafter. The last irrigation date was 4 October.

Residual soil nitrogen. Residual nitrogen in soil samples was determined as total Kjeldahl nitrogen and the analysis was performed by the Analytical Laboratory of the Division of Agriculture and Natural Resources of University of California (Davis, CA), using the standard Kjeldahl procedure with sulfuric acid and digestion catalyst (Issac & Johnson, 1976; Carlson, 1978).

Whitefly numbers. Population dynamics of both adult and immature whiteflies were monitored on a weekly basis throughout the cotton season. Sampling of adult whiteflies was initiated in mid-July and numbers were counted from whiteflies collected with an engine-powered vacuum over the entire 3rd or 4th row in each plot.

Immature whitefly populations were estimated by picking 20 5th main stem node leaves from each of 20 randomly chosen plants (Naranjo, 1996) across the middle four rows within each of the 25 plots. Plant heights in each plot were consistent. Sampling for immatures started in early September when population build-up started. Numbers of nymphs on the underside of each leaf were counted using a microscope.

Table 3. Effect of nitrogen treatments on cotton vegetative growth and seedcotton yield

Nitrogen Treatments (kg N ha^{-1})	Plant height (cm ± SE)	Seedcotton yield (g/10 m ± SE)
0	96.2 ± 0.9 d	1657.6 ± 136.9 a
112	113.9 ± 1.4 c	1612.4 ± 152.8 a
112 plus foliar urea	114.8 ± 1.0 c	1646.0 ± 92.9 a
168	118.7 ± 1.2 b	1573.2 ± 50.4 a
224	123.1 ± 1.0 a	1532.0 ± 48.8 a

Means in columns followed by different letter are significantly different at $P < 0.05$.

Whitefly honeydew drops. Water-sensitive papers (Novartis, Basel, Switzerland) were used to collect whitefly honeydew. Honeydew drops that fell on the papers appeared as easily seen distinct blue spots. The papers (3.3×2.6 cm^2) were secured in a horizontal fashion with a paper clip onto the 10th node (counted from the terminal) petioles of each of five plants chosen randomly across the middle four rows in each plot. After being exposed for about 1 h, the papers

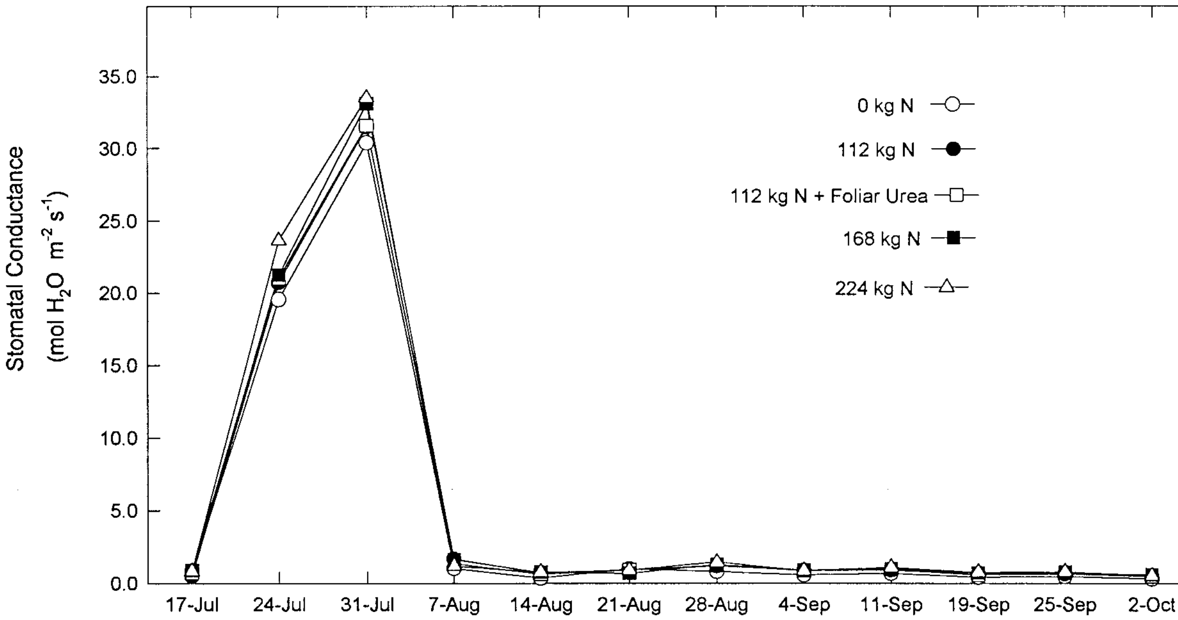

Figure 5. Effect of nitrogen fertilizer treatments on cotton foliar stomatal conductance.

Table 1. Results of regression analysis between numbers[a] of adult whiteflies and amounts of N applied to cotton, or cotton physiological status

Date	Adults and N		Adults and glucose		Adults and fructose		Adults and sucrose		Adults and photosynthetic rate		Adults and stomatal conductance	
	P[b]	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
24 July	0.5949	0.0130	0.6140	0.0112	0.9716	0.0001	0.1809	0.0765	0.6940	0.0069	0.3693	0.0352
31 July	0.2154	0.0650	0.1520	0.0871	0.0625	0.1428	0.1713	0.0798	0.8824	0.0010	0.6707	0.0080
7 Aug.	0.0589	0.1453	0.8391	0.0018	0.8922	0.0008	0.8033	0.0028	0.0675	0.1380	0.9141	0.0005
14 Aug.	0.1363	0.0978	0.0511	0.1555	0.2850	0.0495	0.0554	0.1504	0.9463	0.0002	0.1812	0.0764
21 Aug.	0.3624	0.0378	0.0191	0.2163	0.8579	0.0014	0.0640	0.1413	0.2033	0.0694	0.9212	0.0004
28 Aug.	0.0448	0.1671	0.1819	0.0761	0.9541	0.0002	0.0432	0.1660	0.0017	0.3548	0.6053	0.0118
4 Sep.	0.3220	0.0437	0.5606	0.0149	0.3704	0.0350	0.7791	0.0035	0.0899	0.1200	0.0251	0.1998
11 Sep.	0.5302	0.0166	0.5207	0.0182	0.2893	0.0487	0.8827	0.0010	0.2161	0.0657	0.4287	0.0274
19 Sep.	0.0152	0.2240	0.6523	0.0090	0.7831	0.0034	0.9580	0.0001	0.4409	0.0260	0.4697	0.0230
25 Sep.	0.0380	0.1786	0.0550	0.1509	0.6525	0.0090	0.0890	0.1236	0.0810	0.1265	0.1698	0.0803
2 Oct.	0.0522	0.1605	0.0450	0.1313	0.0205	0.2123	0.2016	0.0699	0.2305	0.0619	0.5605	0.0149
9 Oct.	0.0094	0.2677	0.0482	0.1288	0.1423	0.0912	0.0429	0.1664	0.1471	0.0892	0.3863	0.0328
16 Oct.	0.1625	0.0854	0.0562	0.1303	0.1175	0.1031	0.0295	0.1898				
23 Oct.	0.0576	0.1542	0.5998	0.0122	0.9049	0.0006	0.9420	0.0002				
30 Oct.	0.0550	0.1529	0.4605	0.0239	0.8119	0.0023	0.3715	0.0349				

[a]Numbers of whitefly adult were transformed using $(y + 0.5)^{1/2}$.
[b]Probability.

Table 2. Results of regression analysis between numbers[a] of immature whiteflies and amounts of N applied to cotton, or cotton physiological status

Date	Nymphs and N		Nymphs and glucose		Nymphs and fructose		Nymphs and sucrose		Nymphs and photosynthetic rate		Nymphs and stomatal conductance	
	P[b]	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
4 Sep.	0.0474	0.1652	0.2545	0.0561	0.1606	0.0837	0.0536	0.1525	0.4516	0.0249	0.6503	0.0091
11 Sep.	0.0651	0.1390	0.0003	0.4437	0.0036	0.3135	0.0020	0.3445	0.0081	0.2627	0.1038	0.1109
19 Sep.	0.2501	0.0593	0.0658	0.1396	0.0273	0.1946	0.0240	0.2024	0.2348	0.0608	0.4535	0.0247
25 Sep.	0.0784	0.1274	0.2599	0.0548	0.5960	0.0124	0.5629	0.0148	0.0058	0.2867	0.0612	0.1442
2 Oct.	0.4529	0.0257	0.6747	0.0078	0.2475	0.0577	0.1408	0.0919	0.0156	0.2288	0.0906	0.1195
9 Oct.	0.3893	0.0338	0.1019	0.1121	0.5329	0.0171	0.3066	0.0454	0.1961	0.0716	0.4614	0.0238
16 Oct.	0.1317	0.0967	0.2463	0.0580	0.9890	0.0000	0.3840	0.0331				
23 Oct.	0.0422	0.1727	0.5454	0.0161	0.6173	0.0110	0.6824	0.0074				
30 Oct.	0.0066	0.2810	0.5511	0.0157	0.0433	0.1659	0.1897	0.0736				

[a]Numbers of immature whiteflies were transformed using log $(y + 1)$.
[b]Probability.

were collected and the honeydew drops were counted with the aid of a microscope. Because adult whiteflies were easily disturbed during attachment of the papers to plants, only honeydew drops produced by immature whiteflies were counted. Drops produced by the immature whiteflies were easily differentiated from those produced by the adults according to the drop size (drops produced by adult were several times larger than that produced by immatures). The honeydew droplet counts were made weekly at 16:00 h (Pacific Standard Time) during the peak whitefly population.

Seed cotton yield and plant heights. Seedcotton was harvested on 13 November and 10 December. Open bolls in 10 m of the 5th row within each plot were hand-picked, dried and weighed. Heights of 30 randomly selected plants in the same row were also measured.

Photosynthetic rate and stomatal conductance. Cotton plant photosynthetic rate, stomatal conductance and soluble carbohydrates were monitored throughout the season to identify plant physiological mechanisms of whitefly-cotton interactions. Photosynthetic rates and stomatal conductance were measured every week following fertilization of the plants using a LI-6200 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) equipped with a 1-L stirred cuvette. Measurements were taken near the plant terminals between 11:00 and 13:00 hours when ambient photosynthetic active radiation (PAR) exceeded 1700 μM m^{-2} s^{-2}. One 3rd main stem fully expanded leaf, randomly selected from within the middle four rows of each of the 25 experimental plots, was used for the measurement.

Soluble carbohydrates. Cotton petioles were used for determination of soluble carbohydrates because they contain much vascular tissue and so they may reflect the status of the vascular tissues of the leaf as a whole. Petioles from the 5th main stem leaves were sampled weekly between 15:00 to 16:00 h. The 5th main stem petioles were sampled because the 5th main stem leaves were used for whitefly density estimates (Naranjo, 1996). Ten petioles, from 10 individual plants in the middle four rows of each plot, were excised, wrapped in aluminum foil and immediately dropped into liquid nitrogen to transport to a $-80\,^{\circ}$C freezer. The samples were freeze-dried and ground to powder for assays of soluble carbohydrates.

Carbohydrates were extracted from the petiole powder according to the method of Hendrix (1993). Ten milligrams of the plant tissue powder were extracted three times, each for 8 min, in 1.2 ml of 80% ethanol in an 80 °C water bath. Half a milliliter of the combined extracts was filtered with a centrifugal microfilter tube (CENTREX MF-1.5 with 0.2 μm NYL, Schleicher & Schuell) after mixing with 20 mg of active charcoal to adsorb pigments (Hendrix & Peelen, 1987). The tube was stoppered and vortexed for 2 min and then centrifuged for 5 min to obtain a clear alcohol extract.

Preliminary experiments using HPLC (Hendrix & Wei, 1994) showed that the only sugars in these petioles were glucose, fructose and sucrose (plus the hexitol inositol). To quantify these sugars in the re-

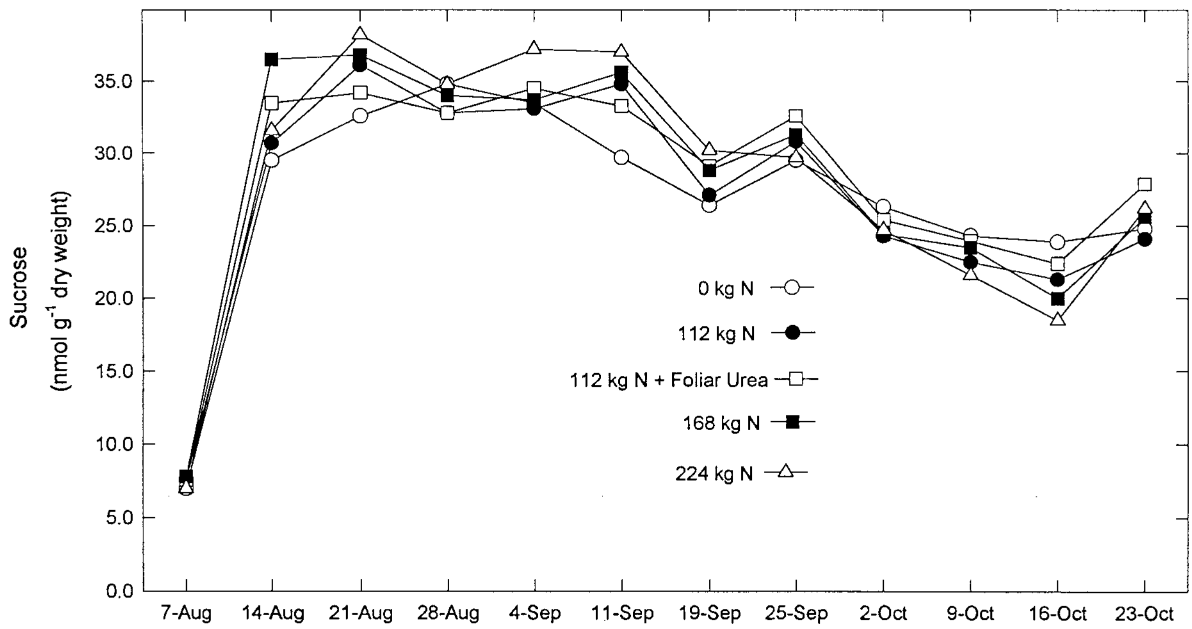

Figure 6. Effect of nitrogen fertilizer treatments on sucrose levels in cotton petioles.

maining samples, a method adopted from those of Hendrix (1993) and Zhao & Oosterhuis (1998) was used. Four 10 μl aliquots from each charcoal-clarified sample were pipetted into separate wells of a microplate and dried at 50 °C for 15 min to remove alcohol. Thereafter, 20 μl of deionized water and 100 μl of glucose-6-P dehydrogenase/iodonitrotetrazolium violet mixture (glucose kit 115A, Sigma Chemical Co.) were added into each well under reduced room illumination. The sample plates were incubated at 37 °C for 15 min, and the glucose concentrations were determined from the absorbance at 492 nm using D-glucose as a standard. Subsequently, 10 μl of phosphoglucose isomerase (PGI enzyme, 0.25 units) was added into each well and re-incubated at 37 °C for another 15 min. The increase in absorbance at 492 nm was used to determine fructose concentrations. Finally, 15 μl of invertase (83 units) was added to each well, and after reincubation of the microplate at 37 °C for a further 15 min, sucrose concentrations were determined by measuring the absorbance at 492 nm.

Statistics. The least significant difference (LSD) test in one-way randomized complete block general linear models (GLM) in SAS (SAS Institute Inc., 1989) was used to analyze the data and separate means. Numbers of whitefly adults from vacuum samples were transformed using the formula $(y + 0.5)^{1/2}$ whereas numbers of immature whiteflies and numbers of honeydew drops were transformed using the formula log $(y + 1)$ before analysis of variance and regression in order to normalize the data (Yee et al., 1998). To determine the relationship between plant physiological factors such as photosynthetic rate, stomatal conductance, sugars and numbers of adult or immature whiteflies, simple regression analyses in SAS (SAS Institute Inc., 1989) was used. Data were averaged over the replications across the blocks for regression analyses.

Results

Residual total nitrogen in soil. Total soil nitrogen in all experimental plots prior to nitrogen treatments was consistent with a mean level of nitrogen around 0.04% ($P > 0.05$).

Whitefly numbers. There was a positive response between nitrogen treatments and numbers of adult or immature whiteflies on most sampling dates during peak population growth (Figures 1 and 2; Tables 1 and 2). Populations began to increase in mid-September and decrease after 23 October. At the highest rate of nitrogen (224 kg ha^{-1}), average adult numbers were 50% greater on 23 October compared to the control

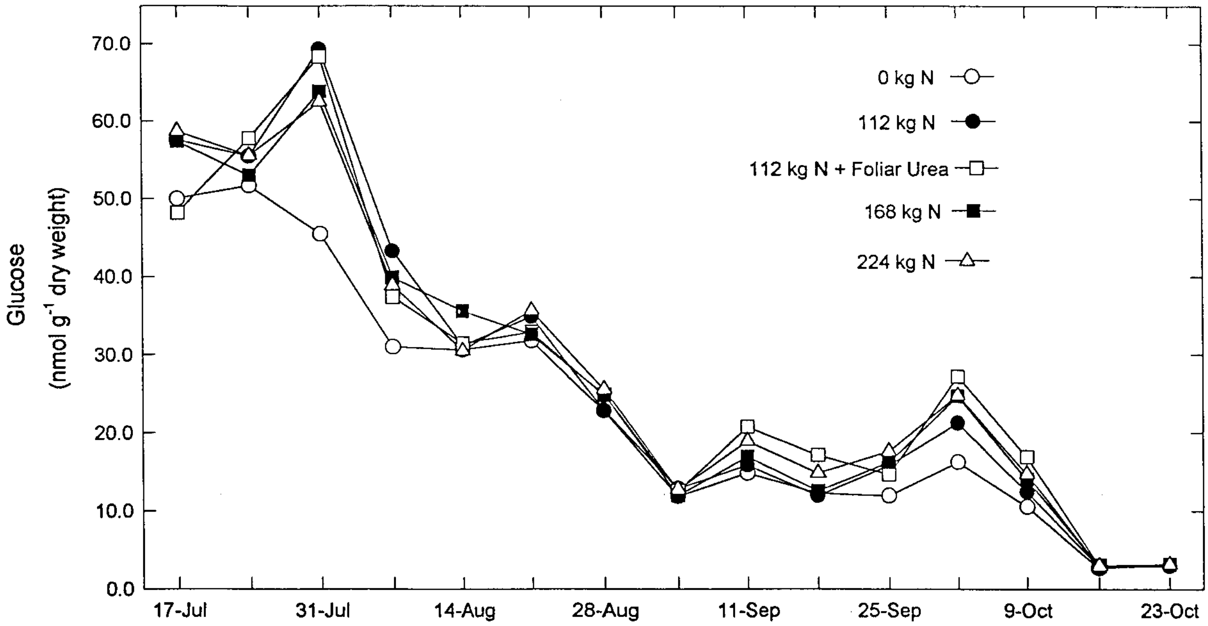

Figure 7. Effect of nitrogen fertilizer treatments on glucose levels in cotton petioles.

(0 kg ha^{-1}) (Figure 1) and immature whitefly numbers were 170% greater than the control at the end of October (Figure 2).

Whitefly honeydew drops. The increased numbers of immature whiteflies resulted in significant increase in honeydew production associated with the nitrogen treatments (Figure 3). Differences between drop numbers among the different nitrogen treatments and the unfertilized control varied as much as 140%.

Plant heights and seed cotton yield. Nitrogen fertilization stimulated increased vegetative cotton growth (Table 3). Plant heights ranged from 96.2 cm for the 0 N treatment to 123.1 cm for the 224 kg N ha^{-1} treatment. The highest rates of nitrogen (168 and 224 kg ha^{-1}) slightly reduced seedcotton yields compared to the other treatments (Table 3), although the differences were not statistically significant ($P > 0.05$).

Photosynthetic rate and stomatal conductance. High photosynthetic rates for all nitrogen treatments occurred from 7 August to 6 September (Figure 4). In general, the application of nitrogen significantly ($P < 0.05$) increased cotton foliar photosynthetic rates throughout the season. Differences in photosynthetic rates among the different levels of applied nitrogen (from 112–224 kg ha^{-1}) were less striking as compared with the control. Results of regression analyses indicated that applied nitrogen was positively correlated ($P < 0.05$) with cotton foliar photosynthetic rates on most of the measuring dates (Table 4).

The effects of nitrogen on stomatal conductance of leaves from different treatments followed similar trends to those observed with photosynthetic rates (Figure 5). In general, applied nitrogen (from 112–224 kg ha^{-1}) significantly increased foliar stomatal conductance relative to the control (0 kg ha^{-1}) throughout the season. Nitrogen applications were also significantly correlated ($P < 0.05$) with foliar stomatal conductance on most measuring dates (Table 4).

Soluble carbohydrates. Applied nitrogen generally increased sucrose levels in petioles before September, with levels in petioles decreasing thereafter (Figure 6). Nitrogen applications also changed glucose levels (Figure 7). In all nitrogen-treated plots, there was a striking increase in glucose levels between 24 July and 14 August, and between 25 September and 9 October. Nitrogen applications generally enhanced fructose levels in cotton petioles in both early and late season (Figure 8). The fructose levels were significantly correlated ($P < 0.05$) with the amount of applied nitrogen on about half of the sampling dates (Table 4).

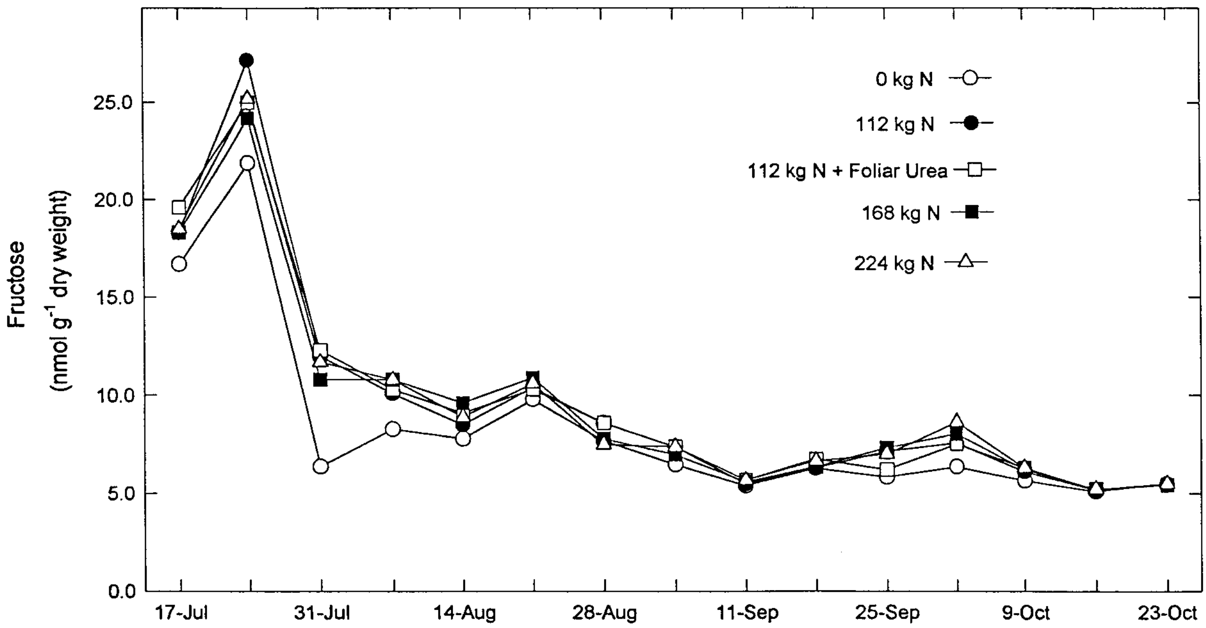

Figure 8. Effect of nitrogen fertilizer treatments on fructose levels in cotton petioles.

Relationship between whitefly numbers and plant physiological parameters. Tables 1 and 2 show the relationships between densities of adult or immature whiteflies and levels of glucose, fructose, sucrose, photosynthetic rate, or stomatal conductance. From 24 July to 19 September, numbers of adult whiteflies were not significantly correlated with glucose levels on all the sampling dates except for 21 August (Table 1). From 25 September to 23 October, with increasing populations of adults, the numbers were significantly correlated with petiole glucose levels on four of the five sampling dates (Table 1). The adult numbers were significantly correlated with the levels of fructose, sucrose, photosynthetic rate or stomatal conductance on only one, four, one and one of the 12–15 sampling dates, respectively (Table 1). Numbers of immature whiteflies were only significantly correlated with levels of glucose, fructose or sucrose on one, three and two of the nine sampling dates, respectively, whereas the numbers were significantly correlated with photosynthetic rates on half of the sampling dates (Table 2). Levels of stomatal conductance were not significantly correlated with the numbers of immature whiteflies on any of the sampling dates (Table 2).

Discussion

Nitrogen fertilizer treatments resulted in significantly increased densities of both adult and immature whiteflies on cotton in the field (Figures 1 and 2; Tables 1 and 2). These results are consistent with findings of other authors for *B. tabaci* on poinsettia, greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), on tomato and cotton aphids, *Aphis gossypii* (Glover), on cotton treated by nitrogen fertilization (Bentz et al., 1995; Jauset et al., 1998; Slosser et al., 1997; Godfrey & Leser, 1999; Godfrey et al., 1999). Higher numbers of the immature whiteflies resulted in significantly increasing honeydew production (Figure 3). A similar relationship between whitefly densities on cotton and honeydew drop production was reported by Yee et al. (1998).

The strong influence of nitrogen treatments on cotton carbohydrate metabolism is shown by the significant effect of enhanced foliar photosynthetic rates and stomatal conductance, and the altered concentrations of glucose, fructose and sucrose in petioles (Figures 4–8). Our results on the beneficial effect of nitrogen on cotton photosynthesis agree with results of Cadena & Cothren (1995) and Bondada et al. (1996). However, Ergle (1936) reported that nitrogen did not affect sugar concentration in cotton as determined using a cuprous titration method. The discrepancy between the results

Table 4. Results of regression analysis between cotton physiological status and amount of nitrogen applied to the cotton

Date	Glucose and N		Fructose and N		Sucrose and N		Photosynthetic rate and N		Stomatal conductance and N	
	P[a]	R^2	P	R^2	P	R^2	P	R^2	P	R^2
24 July	0.5113	0.0195	0.1966	0.0693	0.0010	0.8795	0.2787	0.0528	0.3696	0.0333
31 July	0.0094	0.2003	0.0074	0.2546	0.1167	0.1022	0.0476	0.1636	0.0947	0.1200
7 Aug.	0.0213	0.1872	0.0074	0.0275	0.0901	0.1250	0.5907	0.0133	0.6050	0.0121
14 Aug.	0.5898	0.0131	0.0315	0.1847	0.9474	0.0002	0.0081	0.2777	0.5255	0.0168
21 Aug.	0.2758	0.0535	0.4196	0.0298	0.4207	0.0289	0.0061	0.2898	0.0039	0.3086
28 Aug.	0.1187	0.1061	0.7434	0.0045	0.0015	0.3717	0.0936	0.1169	0.8751	0.0011
4 Sep.	0.6342	0.0104	0.0338	0.1738	0.0029	0.3334	0.0016	0.3602	0.0022	0.3532
11 Sep.	0.1620	0.0862	0.2646	0.0562	0.7332	0.0045	0.0081	0.2539	0.0074	0.2506
19 Sep.	0.3145	0.0459	0.2987	0.0488	0.3016	0.0475	0.0005	0.4263	0.0028	0.3390
25 Sep.	0.0441	0.1715	0.0152	0.2353	0.0383	0.1776	0.0000	0.7654	0.0000	0.5464
2 Oct.	0.0097	0.2539	0.000	0.7221	0.0059	0.2958	0.0009	0.4007	0.0015	0.3703
9 Oct.	0.0900	0.1215	0.0617	0.1478	0.3259	0.0438	0.0000	0.5063	0.0003	0.4272
16 Oct.	0.0046	0.2817	0.0239	0.2035	0.1358	0.0920				
23 Oct.	0.2293	0.0650	0.9126	0.005	0.7157	0.0060				
30 Oct.	0.0971	0.1182	0.2696	0.0551	0.1107	0.1066				

[a]Probability.

of Ergle (1936) and those in our study may be due to the differences in cotton growth environment, cotton varieties and/or differences in analytical methods.

Sucrose, glucose and fructose accounted for over 90% of the ethanol-soluble carbohydrates detected by HPLC in extracts of cotton petioles. These same three sugars are found in phloem sap (Eleftheriou & Hall, 1983a,b) and can be detected in the extrafloral nectar of cotton leaves (D. L. Hendrix, unpubl.). Petiole glucose levels were significantly correlated with densities of whitefly adults during the peak population size (Table 1). However, the phloem sap diet of whiteflies consists of virtually only one sugar, sucrose (Tarczynski et al., 1992). So, the correlation between glucose and whitefly numbers may be due to other factors. Perhaps elevating plant nitrogen also raises plant glucose and it is the nitrogen rather than glucose per se which increases the insect population. Whiteflies are known to impair photoassimilate export from leaves (Lin et al., 2000) which could lead to elevated glucose in leaf petioles. Many other features of a plant's physiology such as quality and quantity of amino acids (Blackmer & Byrne, 1999) and carbohydrate:amino acid ratio (Simpson et al., 1995) are known to be correlated with whitefly population development. It has been suggested that interactions among multiple nutrients be considered in efforts to explain variation in herbivore responses to plant nutrients (Busch & Phelan, 1999).

It is surprising that the applied nitrogen had no effect on seedcotton yield, although vegetative cotton growth was significantly increased (Table 3). Several factors may be involved. First, applied nitrogen increased numbers of both adult and immature whiteflies (Figures 1 and 2). Large whitefly populations can ingest sufficient quantities of plant phloem sap to cause severe reductions in yield (Gerling et al., 1980; Bellows & Arakawa, 1988; Henneberry et al., 1995). Cotton yield has been reported as negatively correlated to numbers of whitefly adults and immatures (Chu et al., 1994; Henneberry, 1995). Thus, the yield-increasing effect of nitrogen may be compromised in part by the large population of whiteflies in this study. Second, in 1998, the cotton season was delayed (planting occurred on 20 May, whereas normal planting is on 20 March) due to low spring soil temperatures. It is likely that the nitrogen treatments further delayed the growing season because there were still many immature bolls on plants treated with nitrogen at season's end (data not shown) when the plants were killed by frost. This maturity-delaying effect of nitrogen on cotton was reported previously (Ebelhar & Welch, 1996). In short season cotton culture, applied nitrogen to cotton resulted in no yield benefit (Boman et al., 1995). Cotton growers in California usually apply about 224 kg N ha^{-1} to their cotton field. Apparently, the growers in 1998 received no benefit from these applications; instead they suffered economic losses from nitrogen fertilizer and application costs and the whitefly outbreaks.

In summary, nitrogen applied to late planted cotton had no effect on seedcotton yield but resulted in increased whitefly numbers and honeydew production. Glucose was the only sugar that was significantly correlated with numbers of whitefly adults during peak populations.

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References

Bellows, T. S. & K. Arakawa, 1988. Dynamics of perimaginal populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Eretmocerus* sp. (Hymenoptera: Aphelinidae) in southern California cotton. Environmental Entomology 17: 225–228.

Bentz, J., J. Reeves, P. Barbosa & B. Francis, 1995. Within-plant variation in nitrogen and sugar content of poinsettia and its effects on the oviposition pattern, survival, and development of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Environmental Entomology 24: 271–277.

Blackmer, J. L. & D. N. Byrne, 1999. Changes in amino acids in *Cucumis melo* in relation to life-history traits and flight propensity of *Bemisia tabaci*. Entomologia Experimentalis et Applicata 93: 29–40.

Blua, M. J. & N. C. Toscano, 1994. *Bemisia argentifolii* (Homoptera: Aleyrodidae) development and honeydew production as a function of cotton nitrogen status. Environmental Entomology 23: 316–321.

Boman, R. K., W. R. Raun, R. L. Westerman & J. C. Banks, 1995. Nitrogen by environment interactions in long-term cotton production. In: P. Dugger & D. Richer (eds), In: Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America, Memphis, TN, pp. 1300–1303.

Bondada, B. R., D. M. Oosterhuis, R. J. Norman & W. H. Baker, 1996. Canopy photosynthesis, growth, yield, and boll ^{15}N accumulation under nitrogen stress in cotton. Crop Science 36: 127–133.

Busch, J. W. & P. L. Phelan, 1999. Mixture models of soybean growth and herbivore performance in response to nitrogen-sulfur-phosphorus nutrient interactions. Ecological Entomology 24: 132–145.

Cadena, J. & J. T. Cothren, 1995. Yield response of cotton to nitrogen, irrigation, and PGR-IV regimes. In: P. Dugger & D. Richer (eds), Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America, Memphis, TN, pp. 1142–1150.

Carlson, R. M., 1978. Automated separation and conductimetric determination of ammonia and dissolved carbon dioxide. Analytical Chemistry 50: 1528–1531.

Chu, C. C., T. J. Henneberry, D. H. Akey, N. Prabhaker & H. H. Perkins, 1994. A study on control action threshold for *Bemisia tabaci* (Gennadius) on cotton. In: P. Dugger & D. Richer (eds), Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America, Memphis, TN, pp. 1239–1241.

Ebelhar, M. W. & R. A. Welch, 1996. Nitrogen rates and mepiquat chloride effects on cotton lint yield and quality. In: P. Dugger & D. Richer (eds), Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America Memphis, TN, pp. 1373–1378.

Eleftheriou, E. P. & J. L. Hall, 1983a. The extrafloral nectaries of cotton. I. Fine structure of the secretory papillae. Journal of Experimental Botany 34: 103–119.

Eleftheriou, E. P. & J. L. Hall, 1983b. The extrafloral nectaries of cotton. II. Cytochemical localization of ATPase and Ca^{2+}-binding sites, and selective osmium impregnation. Journal of Experimental Botany 34: 1066–1079.

Ergle, D. R., 1936. Carbohydrate content of cotton plants at different growth periods and the influence of fertilizer. Journal of American Society of Agronomy 30: 951–959.

Fisher, D. B., J. P. Wright & T. E. Mittler, 1984. Osmoregulation by the aphid *Myzus persicae*: a physiological role for honeydew oligosaccharides. Journal of Insect Physiology 30: 387–393.

Gerling, D., U. Motro & R. Horowitz, 1980. Dynamics of *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae) attacking cotton in the coastal plain of Israel. Bulletin of Entomological Research 70: 213–219.

Godfrey, L. D. & Leser, J. F., 1999. Cotton aphid management – status and needs In: P. Dugger & D. Richer (eds), Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America, Memphis, TN, pp. 37–40.

Godfrey, L. D., K. Keillor, R. B. Hutmacher & J. Cisneros, 1999. Interaction of cotton aphid population dynamics and cotton fertilization regime in California cotton. In: P. Dugger & D. Richer (eds), Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America, Memphis, TN, pp. 1008–1011.

Hendrix, D. L., 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. Crop Science 33: 1306–1311.

Hendrix, D. L., 1999. Sugar composition of cotton aphid and silverleaf whitefly honeydews. In: P. Dugger & D. Richer (eds), Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America, Memphis, TN, pp. 47–51.

Hendrix, D. L. & K. K. Peelen, 1987. Artifacts in the analysis of plant tissues for soluble carbohydrates. Crop Science 27: 710–715.

Hendrix, D. L. & Y. A. Wei, 1994. Bemisiose: an unusual trisaccharide in *Bemisia* honeydew. Carbohydrate Research 253: 329–334.

Hendrix, D. L., Y. Wei & J. E. Leggett, 1992. Homopteran honeydew sugar composition is determined by both insect and plant species. Comparative Biochemistry & Physiology 101B: 23–27.

Hendrix, D. L. & M. E. Salvucci, 2000. Isobemisiose: an unusual trisaccharide abundant in the silverleaf whitefly, *Bemisia argentifolii*. Journal of Insect Physiology (in press).

Henneberry, T. J., D. L. Hendrix, H. H. Perkins, S. E. Naranjo, H. M. Flint, D. Akay, L. F. Jech & R. A. Burke, 1995. *Bemisia argentifolii* (Homoptera: Aleyrodidae) populations and relationships to sticky cotton and cotton yields. Southwestern Entomologist 20: 255–271.

Henneberry, T. J., D. L. Hendrix, H. H. Perkins, L. F. Jech & R. A. Burke, 1996. *Bemisia argentifolii* (Homoptera: Aleyrodidae) honeydew sugars and relationships to sticky cotton. Environmental Entomology 25: 551–558.

Issac, R. A. & W. C. Johnson, 1976. Determination of total nitrogen in plant tissue, using a block digester. Journal of Association of Official Analytical Chemists 59: 98–100.

Jasson, R. K. & Z. Smilowitz, 1986. Influence of nitrogen on population parameters of potato insects: abundance, population growth, and within-plant distribution of the green peach aphid, *Myzus persicae* (Homoptera: Aphididae). Environmental Entomology 15: 49–55.

Jauset, A. M., M. J. Sarasua, J. Avilla & R. Albajes, 1998. The impact of nitrogen fertilization of tomato on feeding site selection and oviposition by *Trialeurodes vaporariorum*. Entomologia Experimentalis Applicata 86: 175–182.

Joern, A. & S. T. Behmer, 1997. Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). Oecologia 112: 201–208.

Lin, T. B., S. Wolf, A. Schwartz & Y. Saranga, 2000. Silverleaf whitefly stress impairs sugar export from cotton source leaves. Physiologia Plantarum 109: 292–297.

Naranjo, S. E., 1996. Sampling *Bemisia* for research and pest management applications. In: D. Gerling & R. T. Mayer (eds), *Bemisia*: 1995, Taxonomy, Biology, Damage, Control & Management, pp. 209–224.

Perkins Jr., H. H., 1986. Whitefly honeydew in US cottons: update on methods for detecting and processing contaminated cottons. In: P. Dugger & D. Richer (eds), Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America, Memphis, TN, pp. 106–107.

Rhodes, T. D., P. C. Croghan & A. F. G. Dixon, 1997. Dietary sucrose and oligosaccharide synthesis in relation to osmoregulation in the pea aphid, *Acrythosiphon pisum*. Physiological Entomology 22: 373–379.

Rubeiz, I. G., M. Aslam, H. Chahine & A. Imad, 1995. Whitefly populations in greenhouse cantaloupe as affected by poultry manure and inorganic nitrogen fertilizer application. Biological Agriculture & Horticulture 12: 277–281.

SAS Institute Inc.,1989. SAS/STAT User's Guide, Version 6, Cary, NC.

Simpson, S. J., J. D. Abisgold & A. E. Douglas, 1995. Response of the pea aphid (*Acrythosiphon pisum*) to variation in dietary levels of sugar and amino acids: the significance of amino acid quality. Journal of Insect Physiology 41: 71–75.

Slosser, J. E., W. E. Pinchak & D. R. Rummel, 1997. Cotton aphid response to nitrogen fertility in dryland cotton. In: P. Dugger & D. Richer (eds), Proceedings of Beltwide Cotton Research Conference. National Cotton Council of America, Memphis, TN, pp. 1051–1053.

Tarczynski, M. C., D. N. Byrne & M. B. Miller, 1992. High performance liquid chromatography analysis of carbohydrates of cotton phloem, sap and honeydew produced by *Bemisia tabaci* feeding on cotton. Plant Physiology 98: 753–756.

Wei, Y. A., D. L. Hendrix & R. Nieman, 1996. Isolation of a novel tetrasaccharide, bemisiotetrose, and glycine betaine from silverleaf whitefly honeydew. Journal of Agriculture and Food Chemistry 44: 3214–3218.

Wei, Y. A., D. L. Hendrix & R. Nieman, 1997. Diglucomelezitose, a novel pentasaccharide in silverleaf whitefly honeydew. Journal of Agriculture and Food Chemistry 45: 3281–3486.

White, T. C. R., 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia 63: 90–105.

Yee, W. L., N. C. Toscano, C. C. Chu, T. J. Henneberry & R. L. Nichols, 1996. *Bemisia argentifolii* (Homoptera: Aleyrodidae) action thresholds and cotton photosynthesis. Environmental Entomology 25: 1267–1273.

Yee, W. L., N. C. Toscano, D. L. Hendrix & T. J. Henneberry, 1998. Effect of insecticide applications on *Bemisia argentifolii* (Homoptera: Aleyrodidae) densities and honeydew production. Environmental Entomology 27: 22–32.

Zhao, D. & D. Oosterhuis, 1998. Physiological and yield responses of shaded cotton to the plant growth regulator PGR-IV. Journal of Plant Growth Regulator 17: 47–52.